Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Suppl 2, 2013 ISSN - 0974-2441

Research Article

ISOLATION OF MANGIFERIN FROM LEAVES OF *MANGIFERA INDICA* L. VAR ALPHONSO

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Received: 19 February 2013, Revised and Accepted: 21 March 2013

ABSTRACT
Mango is rich source of biologically active compound mangiferin. In the present study, mangiferin was isolated from the leaves of *Mangifera indica* L. var Alphonso family Anacardiaceae and isolated compound was found to be same as evidence by UV, IR and ^{1}H NMR studies

Keywords: Mangifera indica, Anacardiaceae, Isolation, Ultraviolet spectroscopy, Fourier transform spectroscopy, and ^{1}H-NMR.

INTRODUCTION

Tropical trees, of which *Mangifera indica* is the only one well known horticulturally, and is cultivated throughout the tropics for its fruit, being naturalized in many regions. It is finer varieties one of the most delicious of all tropical fruits. Several pharmacological activities of mango extract have been reported including anti-inflammatory, antioxidant, antiallergic, anthelmintic and antiamoebic.

Phytochemical studies on various parts of *M. indica* revealed that it contains many phenolic compounds and Mangiferin, a xanthone glycoside. Mangiferin has been reported to possess antioxidant[1], antitumor[2], immunomodulatory[2], antiHIV[2], antiviral[3], inhibit bowel carcinogenesis[4], antipyretic activity[5], anticancer[6], antidiabetic[7], anthelmintic[8], antimicrobial[9], antibacterial[10] and neuroprotective activity[11].

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTIFICATION

The leaves of the plant *Mangifera indica* Linn. var Alphonso selected was collected from Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai District, India and was authenticated by Dr.P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, TamilNadu, India, and Dr. T. Arumugam Professor – Horticulture, AC and RI, TamilNadu Agricultural University, Madurai- 625 104, TamilNadu, India.

Equipments

Soxhelt apparatus, Buchi Rotovapor, melting point apparatus and UV Spectroscopy.

Isolation of Mangiferin:[12]

The leaves of *Mangifera indica* L. var Alphonso (Anacardiaceae) were collected, shade dried and powdered. The powderd plant materials (100g) were defatted with petroleum ether (60-80^{0}C). Defatted powdered leaves were extracted by soxhelt with required quantity of ethanol for 21h and concentrated under reduced pressure to yield semisolid mass.

The concentrated mass were resuspended in 50ml of 50% ethanol then partitioned with 100ml dichloromethane for 4 times. The aqueous ethanolic phase was hydrolysed by reflux with 2N sulphuric acid at pH 3 for an hour with continuous stirring. After cooled to room temperature, it was partitioned with 100 ml ethyl acetate for 3 times. Subsequently, the combined ethyl acetate layer was dried at 40^{0}C using a vacuum rotary evaporator. The dried ethyl acetate fraction was dissolved in ethanol and left in a refrigerator (4-8^{0}C) over night. After that the precipitate came out and was isolated by filtration. For crystallization, the precipitate was dissolved in 70% aqueous ethanolic solution and left in a refrigerator (4-8^{0}C) over night. Lastly, the pale yellow needle-shaped crystals of mangiferin were isolated and dried. The isolated compound was further characterized using TLC, melting point, UV/VIS, FTIR and ^{1}H NMR spectrophotometry compered to reference standard mangiferin.

RESULTS

The yield of mangiferin obtained was 1.2g. The isolated compound was further characterized using TLC, melting point, UV/VIS, FTIR and ^{1}H NMR spectrophotometry compared to reference standard mangiferin.

TLC OF THE ISOLATED COMPOUND[13]

Identification of the isolated compound were performed on TLC analysis compared to reference standard mangiferin.

Adsorbent Precoated silica gel 60 F_{254} plate.

Mobile phase Ethyl acetate: formic acid: glacial acetic acid: water 100: 11: 11: 26

Sample preparation 0.05% w/v of sample was prepared and 10µl applied on TLC plate.

Detector Ferric chloride reagent spray.

Rf value : 0.49

Melting point of isolated mangiferin was 292^{0}C

Ultraviolet spectroscopy[14]

The isolated compound was prepared at concentration of 40µg/ml by dissolving in ethanol. Scanning of the isolated compound was performed at the wavelength range of 200-400 nm and compared with reference standard.

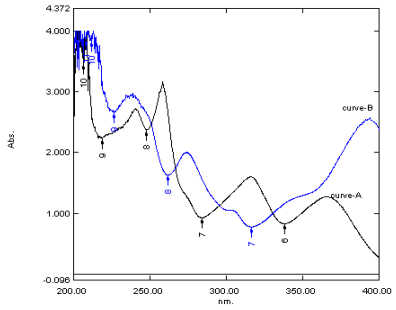

Figure 1: UV ABSORBANCE OF ISOLATED COMPOUND AND THE SHIFT BY ADDITION OF NaOH

Curve-A solvent used is 95% ethanol. Wave length Maxima↑ are240, 258, 316, 364nm. (Minimum↓ are 218, 247, 284, 338nm).

Curve-B 95% ethanol + 2drops of 2N NaoH. Relative intensities of absorbance at the Maxima↑ are 394, 273, 238, 212nm. (Minimum↓ are 316, 261, 226, 208nm).

Fourier transform spectroscopy[15]

Introduction- Model- Buck-Scientific

Method- potassiumchloride pellet method

IR data of isolated compound was compared with the reference standard of mangiferin.

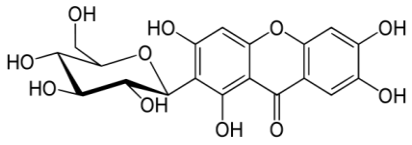

Figure 2: STRUCTURE OF MANGIFERIN

Molecular formula-$C_{19}H_{18}O_{11}$

Table 1:

S.No	Absorbance(cm^{-1})	Groups
1	3367	Phenol O-H stretch
2	2918.38, 2849.99	Aliphatic C-H stretch
3	1670	Keto C=O stretch
4	1624.26	Aromatic C=C ring stretch
5	1253.98	Ar-O-Ar ether C-O-C stretch
6	1051.52	RCH_2OH O-H stretch
7	828.41	Tetra substituted aromatic

^{1}H-NMR data's of isolated compound[16]

^{1}H-NMR(DMSO-*d*6) δ: 13.76 (s, 1H, 1 –OH), 10.55 (s, 1H, 6, 7-OH), 9.86 (s, 1H, 3-OH), 7.38 (s, 1H, 8H), 6.37 (s, 1H, 4H)

Discussion and conclusion

The pharmacologically active mangiferin could be isolated using uncomplicated method, ethanol was used as solvent to obtain crude extract. Partitioning with dichloromethane to eradicate some pigment matters and non-polar compounds contained in the plant then purified them. Consequently, hydrolyzation was achieved at pH3 to remove some impurities such as O- glycosides before partitioning with ethyl acetate. After drying the ethyl acetate fraction was dissolved in ethanol and left at cool temperature (4-8^{0}C) over night. Mangiferin precipitated. Finally, the precipitated mangiferin was recrystallized in 70% aqueous ethanolic solution and left in a refrigerator (4^{0}C) over night and the pale yellow needle shaped crystals of mangiferin was isolated and dried. It showed identical TLC chromatography and UV, IR, NMR spectrum to reference standard mangiferin.

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